View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

Symposium: Viral Entry, Budding and Replication at Membranes

66-Symp
Sculpting and Subversion of Membranes during Poliovirus in Assembly of RNA Replication Complexes and in Egress
Karla Kirkegaard.
Dept. of Microbiology & Immunology, Stanford University, Standford, CA, USA.
Infection with many positive-strand RNA viruses dramatically remodels cellular membranes, resulting in the accumulation of double-membraned vesicles that resemble cellular autophagosomes. In this study, a single protein encoded by poliovirus, 3AB, reconstituted onto synthetic unilamellar liposomes is shown to be sufficient to induce the formation of double-membraned liposomes via the invagination of single-membraned liposomes. The ability of a single viral protein to produce structures of similar topology to cellular autophagosomes should facilitate understanding of both cellular and viral mechanisms for membrane remodeling. What is the purpose for the virus of constructing these structures? The cellular process of autophagy is involved not only in the generation of intracellular structures for the replication of viral RNA, but also for the nonlytic spread of viruses between cells, On the cytoplasmic face of the vesicles generated by membrane proliferation, RNA replication complexes are establlshed. These complexes include the viral RNA-dependent RNA polymerase, a 52-kd protein that can oligomerize into two-dimensional arrays likely to form replicative surfaces on these membranes. The double-membraned topology of the vesicles is then utilized for the 'unconventional secretion' of cytoplasmic viruses from the cell without lysis. Membrane dynamics during Dengue virus will also be discussed, in which the assembly of viral particles is critically dependent on the process of autophagy

67-Symp
Class II Membrane Fusion Proteins in Viral and Cellular Fusion Events
Félix Rey[1], Benjamin Podbilewicz[2], Jimena Perez-Vargas[1], Thomas Krey[1].
[1]Pasteur Institute, Paris, France, [2]Technion, Haifa, Israel.
Class II proteins are viral membrane fusogenic molecules folded essentially as beta-sheet and having an internal fusion peptide. In particular, they lack the characteristic central alpha-helical coiled coil present in the post-fusion conformation of all other viral fusion proteins. The regular, symmetric enveloped viruses that have been studied so far, such as flaviviruses and alphaviruses, have been shown to have class II fusion proteins, which in their pre-fusion conformation make an icosahedral shell surrounding the viral membrane. The negative-stranded RNA viruses of the bunyavirus family - which have been shown to display a regular icosahedral glycoprotein shell at their surface, have also been predicted to have class II fusion proteins. We have recently identified the rubella virus fusion protein as class II, although the virus particles appear pleomorphic. In spite of the lack of sequence conservation, the available structures indicate that class II proteins have evolved from a distal, ancestral gene. We have now discovered that the cellular fusion protein Eff-1, involved in syncytium formation during the genesis of the skin in nematodes (C. elegans) and in other multicellular organisms, are also folded as a class II viral fusion proteins, thereby indicating common ancestry. My talk will discuss the implications of this finding, which highlights the intricate exchange of genetic information that have taken place between viruses and cells during evolution. It also suggest a mechanism for the cell-cell fusion process, which ahs not been studied so far.

68-Symp
The HIV-1 Capsid: Assembly and Restriction by TRIM5α
Barbie K. Ganser-Pornillos[1], Owen Pornillos[1], Viswanathan Chandrasekaran[2], Anchi Cheng[3], Wesley I. Sundquist[2], Mark Yeager[1,3].
[1]University of Virginia, Charlottesville, VA, USA, [2]University of Utah, Salt Lake City, UT, USA, [3]The Scripps Research Institute, La Jolla, CA, USA.
The mature HIV-1 capsid packages the viral genome and facilitates the reverse transcription step in early stages of the viral replication cycle. The capsid is a cone-shaped fullerene shell composed of ~1,500 copies of the virally encoded CA protein. The subunits form a two-dimensional array of CA hexamers that is closed by incorporation of 12 CA pentamers. Despite the locally symmetric arrangement of the subunits, the capsid particle itself is non-symmetric. Therefore, we have used a "hybrid methods" strategy to study the capsid architecture. Initially, we used electron cryomicroscopy (cryoEM) to obtain low-resolution views of the quaternary interactions that mediate hexamer formation by analyzing capsid-like structures formed *in vitro*. Docking atomic resolution structures of CA subdomains into the cryoEM map yielded a Cα model, which we used as a template to design disulfides that stabilized the hexamer, and the quasi-equivalent pentamer. The structures of the building blocks were then solved by X-ray crystallography. By comparative analysis of all the available structures, we identified conformational switches that facilitated the formation of a non-symmetric capsid. The structural and mechanistic elements were then combined computationally into an atomic model for the complete capsid.
TRIM5α is a cellular innate immune factor that interferes with retroviral capsid function. TRIM5α directly binds the capsid, but its pair-wise affinity for the CA subunit is extremely weak. We have found that TRIM5α assembles into a net-like lattice that matches the symmetry and spacing of the capsid lattice. This suggests that recognition of the HIV-1 capsid by TRIM5α occurs through lattice-lattice interactions, and that the weak pair-wise affinity of TRIM5α for the CA subunit is amplified by avidity. These studies support a model wherein TRIM5α constitutes a soluble surveillance mechanism in the cell that intercepts and disables the incoming viral capsid.

69-Symp
The ESCRT Pathway in HIV Budding and Cell Division
Wesley I. Sundquist.
Biochemistry, University of Utah School of Medicine, Salt Lake City, UT, USA.
The Endosomal Sorting Complexes Required for Transport (ESCRT) pathway mediates intraluminal endosomal vesicle formation, budding of HIV-1 and other enveloped viruses, and the final abscission step of cytokinesis in mammals and archaea. I will review our current understanding of the roles of different ESCRT factors in HIV budding and abscission. In particular, I will describe our experiments aimed at understanding how the filament-forming ESCRT-III subunits and the VPS4 ATPase assemble and function in membrane fission.

Symposium: Chromosome Packaging, Processing, and Dynamics

70-Symp
Insights into DNA Packaging and Gene Expression from Computer Simulations
Wilma Olson.
Dept. of Chemistry/Chemical Biology, Rutgers, the State University of New Jersey, Piscataway, NJ, USA.
Within the nucleus of each cell lies DNA - an unfathomably long, twisted, and intricately coiled molecule - segments of which make up the genes that provide the instructions that a cell needs to operate. As we near the 60th anniversary of the discovery of the DNA double helix, crucial questions remain about how the physical arrangement of the DNA in cells affects how genes work. For example, how a cell stores the genetic information inside the nucleus is complicated by the necessity of maintaining accessibility to DNA for genetic processing. In order to gain insight into the roles played by various proteins in reading and compacting the genome, our group has developed new methodologies to simulate the dynamic, three-dimensional structures of long, fluctuating, protein-decorated strands of DNA. Our a priori approach to the problem allows us to determine the effects of individual proteins and their chemical modifications on overall DNA structure and function. The talk will cover our recent treatment of the communication between regulatory proteins attached to precisely constructed stretches of chromatin. Our simulations account for the enhancement in communication detected experimentally on chromatin compared to protein-free DNA of the same chain length as well as the critical roles played by the cationic 'tails' of the histone proteins in this signaling. The states of chromatin captured in the simulations offer new insights into the ways that the DNA, histones, and regulatory proteins contribute to long-range communication along the genome.

71-Symp
Molecular Recognition of the Nucleosome by Chromatin Enzymes and Factors
Song Tan, Ravindra Makde, Joseph R. England, Jiehuan Huang, Hemant Yennawar.
Penn State University, University Park, PA, USA.
Eukaryotic DNA is packaged in the nucleus as chromatin, and the fundamental repeating unit of chromatin is the nucleosome. The packaging of histone proteins and DNA into nucleosomes has important consequences for gene regulation, and dozens of chromatin enzymes and factors that target the nucleosome for gene activation and repression have been discovered. However, the